Investigations on the Toxic & Teratogenic Effects of GRAS Substances on the Developing Chick Embryo **Lactose** No Date

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Investigations on the Toxic and Teratogenic Effects of GRAS Substances on the Developing Chick Embryo.[1]

Lactose

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[1]Report of investigations conducted under Contract No. 72-343 with the Food and Drug Administration, PHS, DHEW.

General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table i. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, solubility of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by auto claving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway Model 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 18 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced to 98.5° F dry bulb reading and humidity was increased to a 90° F wet bulb reading. Upon hatching (22nd day) chicks were examined for abnormalities and samples were cleared and alizarin stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, muscle, bursa, brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embeded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in duplicate and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected controls and untreated control groups of eggs were used with each experiment. In some cases, extra trials were conducted to provide embryos for examination at critical doses of the test substances in order to further evaluate teratogenic response and obtain additional data on the nature of embryonic defects.

Data obtained from the experiments (except that from the range finding studies) was transferred to data sheets provided (FDH form 2572, 2572a and 2572b) and submitted to FDA for statistical analysis. Nine types of data summaries including 2 statistical treatments of the data were provided by FDA on the data submitted. The results presented and interpretations made are largely based on these data summaries.

Table i

FDA Project Test Substances

Test Substance and Identification		Compound No.
1. Lactose, Edible Formost Dairies, Inc. Appleton, Wisc.		000063423
2. Propyl Gallate Lot 337		000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 965102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3167 73(C)		000134032
4. Sodium Erythorbate F.C.C. Lot No. 834072 FDA 3167 73(C) Hoffmann-LaRoche, Nutley, N. J.		977052064
5. Oil Nutmeg NF, East Indian Fritzsche Dodge & Olcott, Inc. 71-28 New York, N. Y.		MX 8008455
6. Zinc Sulfate - Rayon Lot # 2132R1 Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. Monohyd.	007733020 007446197
7. Stannous Chloride, AR $2H_2O$ Mallinckrodt Chemical Works St. Louis, Mo.		007772998
8. Talc USP #141, Whittaker, Clark and Daniels, Inc.		010101390
9. Carob Bean Gum FDA 71-14		PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 Witco Chemical Organics Division New York, N. Y. EMCOL D70-30C		977051323

General Discussion and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 96 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and carob bean gum. Moderate toxicity was encountered with sodium ascorbate, sodium erythorbate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table ii

Comparison of Ten Substances Tested
for Toxicity and Teratology

Substance Tested	LC_{50} via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	84 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micromelia, torticollis, celosomia.
Phosphated Mono- and Di-Glycerides	>3000 mgs./kg.	Ascites, anophthalmia, brachygnathia.

I. LACTOSE

Specific Protocol:

Lactose is highly water soluble and maximum solubility was achieved at 200 mgs./ml. The solution was autoclaved for sterilization without problems. This solution and dilutions of it was injected into eggs at 100 µl volume. Five doses of lactose were tested at both 0 and 96 hours of incubation and via both air cell and yolk routes of administration.

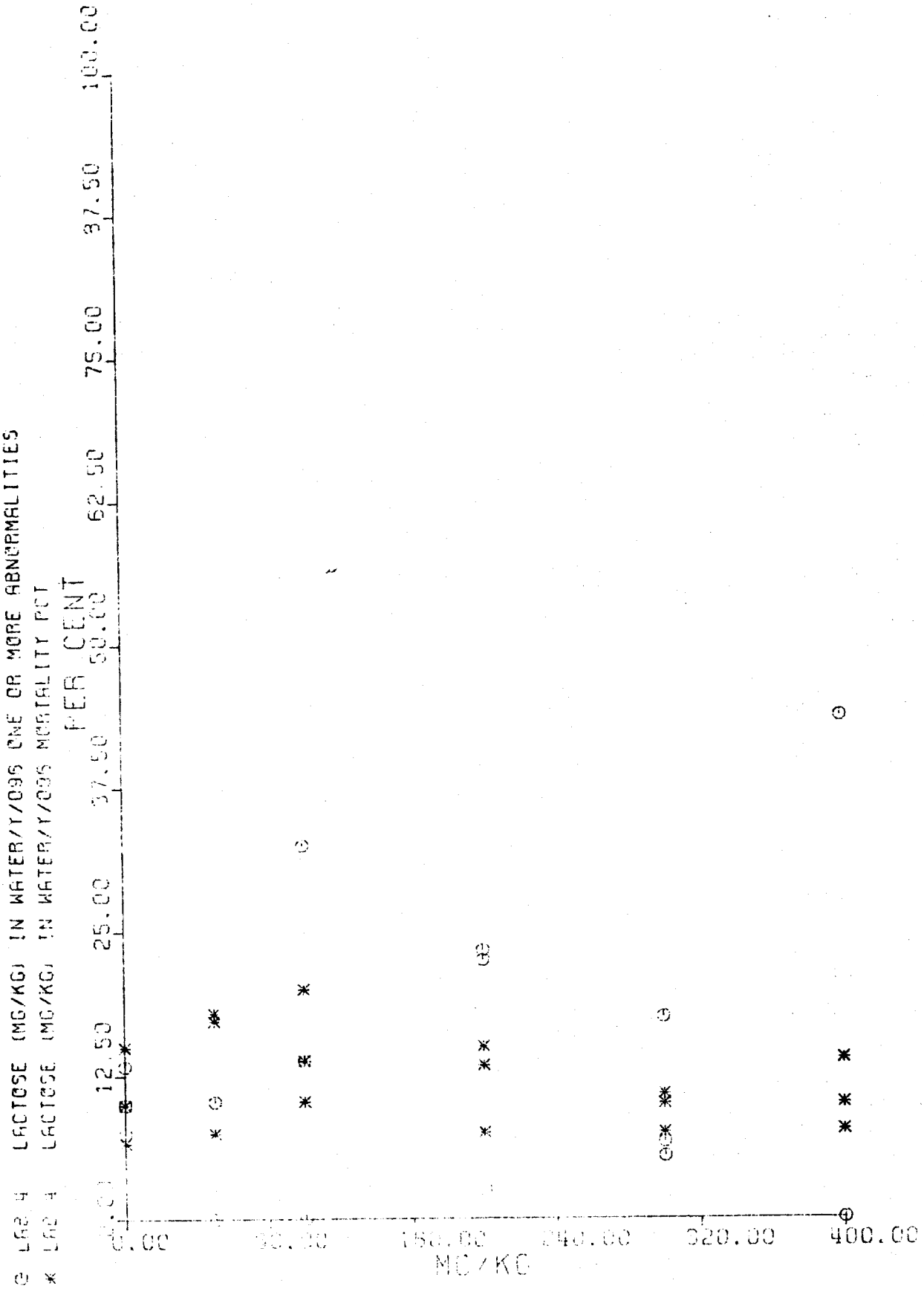
Results:

The data for lactose is presented in Tables 1-4. Percent mortality was not significantly increased by any of the dose levels of lactose. The highest dose of 20 mgs./egg was nontoxic and corresponded to the maximum amount of this substance soluble in water that could be injected at 100 µl. volume. The percent of abnormal chicks hatched was not significantly increased via air cell at 0 hrs., air cell at 96 hrs. or yolk injection at 0 hrs. However, a significant increase in abnormal chicks was observed at the 5 mg./egg dose when lactose was given by yolk injection at 96 hrs. Larger doses (10, 15 and 20 mgs./egg) of lactose did not significantly increase the percent of abnormal chicks hatched and therefore the increase observed at 5 mgs./egg was in all probability a chance event without real meaning. The percent of embryos showing H-S-V-L (head, skeletal, visceral or limb) abnormalities was not significantly increased by any dose of lactose or under any of the conditions tested. No specific gross abnormalities or histopathological findings appeared to be related to either dose or test conditions.

Discussion:

It seems clear that lactose was nontoxic under the conditions of these experiments and it is very doubtful that it has any teratological effect on the developing chick embryo at levels up to 20 mgs./egg. Solubility limits and physical limits on the volume of solution that can be injected preclude testing larger amounts.

About 6 percent of the control chicks exhibited one or more abnormalities. A wide variety of specific abnormalities were found but none of them appeared in the lactose treated groups in any number that would make them appear treatment related except that the appearance of ascites seemed to be greater when embryos were injected at 96 hrs. via the yolk with larger doses of lactose. This was considered to be a mild embryo toxic response but did not interfere with activity or survival. No dose approaching an LC_{50} could be administered.


LACTOSE (MG/KG) IN WATER/Y/035 ONE OR MORE ABNORMALITIES
LACTOSE (MG/KG) IN WATER/Y/035 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
0.00
400.00
MG/KG

LAB 4 LACTOSE (MG/KG) IN WATER/A/000 ONE OR MORE ABNORMALITIES
LAB 4 LACTOSE (MG/KG) IN WATER/A/000 MORTALITY PCT
PER CENT
12.50
25.00
50.00
62.50
75.00
87.50
100.00

Lab 4

LAC 4 LACTOSE (MG/KG) IN WATER/A/096 ONE OR MORE ABNORMALITIES
LAC 4 LACTOSE (MG/KG) IN WATER/A/096 MORTALITY PCT
PER CENT
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00

LAB 4 LACTOSE (MG/KG) IN WATER/Y/000 ONE OR MORE ABNORMALITIES
LAB 4 LACTOSE (MG/KG) IN WATER/Y/000 MORTALITY PCT
PER CENT
.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
400.00

Lab 4

Table 1

DATA SUMMARY

Lactose in Water
via Air Cell at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	439	6.83	6.15	1.59
Solvent	None	110	11.81	4.54	0
50.0	2.5	110	12.72[1]	0.90	0
100.0	5.0	110	10.00	7.27	1.81
200.0	10.0	109	9.17	2.75	1.83
300.0	15.0	108	9.25	8.33[2]	1.85[3]
400.0	20.0	109	9.17	2.75	0.95

[1] NS
[2] NS
[3] NS
[4] Slope is negative
[5] F (Cal) < F(.05)

Table 2

DATA SUMMARY

Lactose in Water
via Air Cell at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	439	6.83	6.15	1.59
Solvent	None	108	5.55	3.70	0.92
50.0	2.5	109	10.09	3.66	1.83
100.0	5.0	110	10.90[1]	7.27[2]	1.81
200.0	10.0	109	3.66	3.66	1.91[3]
300.0	15.0	110	3.63	2.72	1.81
400.0	20.0	109	4.58	6.42	1.83

[1] NS
[2] NS
[3] NS
[4] Slope is negative
[5] Same as 4

Table 3

DATA SUMMARY

Lactose in Water
via Yolk at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	439	6.83	6.15	1.59
Solvent	None	109	33.94	2.75	3.66
50.0	2.5	109	28.44	5.50	0
100.0	5.0	109	21.10[1]	2.75	0
200.0	10.0	109	33.02	5.50	4.58[3]
300.0	15.0	110	27.27	2.72	0.90
400.0	20.0	106	32.07	7.54[2]	0.94

1 NS
2 NS
3 NS
4 F (Cal) <F (.05)
5 Same as 4

Table 4

DATA SUMMARY

Lactose in Water
via Yolk at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	439	6.83	6.15	1.59
Solvent	None	110	10.90	10.00	1.81
50.0	2.5	108	13.88	5.55	0.92
100.0	5.0	108	14.81[1]	18.51[2]	1.85
200.0	10.0	110	11.81	15.45	0
300.0	15.0	107	9.34	10.28	3.73[3]
400.0	20.0	105	10.47	16.19	0

1 NS

2 Difference from lowest test dose is significant

3 NS

4 Slope is negative

5 F (Cal) $<$ F (.05)